Anti-ATL3 Antibody [PSH02-47]

HA721824

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 61 kDa
Clone number:	PSH02-47

Description: ATLs maintain the ER tubular network via homotypic fusion. ATLs have a conserved domain structure consisting of a globular G domain, a three-helix bundle, two transmembrane domains, and an amphipathic helix. The ATL fusion cycle consists of two ATL monomers in opposing membranes binding GTP, which induces trans G domain dimerization and a crossing over of the three-helix bundle. Crossover and subsequent insertion of the amphipathic helix into the lipid bilayer triggers lipids to mix for fusion. Lastly, GTP is hydrolyzed driving the dimer to disassembly and resetting the fusion machinery. While most of the human ATL protein structure is conserved between paralogs, the proteins have non-conserved N- and C-termini with the C-termini of ATL1 and ATL2 being autoinhibitory. ATL1 has been shown to interact with a range of proteins including spastin and REEP1, with spastin enhancing ATL1 fusion activity in vitro. ATL1 and ATL2 have also been observed as interacting with ER protein TMCC3, and ATL3 with nonstructural viral proteins, however it is not currently known how these interactions modulate protein function.

Immunogen: Recombinant protein within human ATL3 aa 1-488 / 541.

Positive control: HeLa cell lysate, Jurkat cell lysate, A549 cell lysate, HepG2 cell lysate, COS-1 cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, Neuro-2a cell lysate, PC-12 cell lysate, L6 cell lysate, mouse liver tissue lysate, rat liver tissue lysate, mouse liver tissue, rat liver tissue, HeLa, NIH/3T3.

Subcellular location: Endoplasmic reticulum membrane.

Database links: SwissProt: Q6DD88 Human | Q91YH5 Mouse | Q0ZHH6 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
IHC-P	1:1,000
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

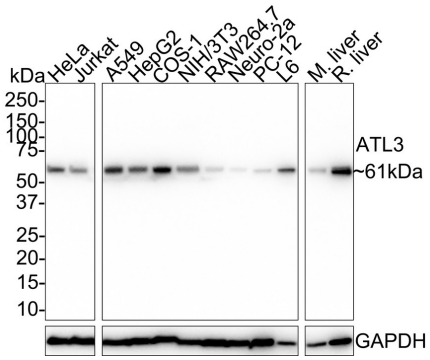

Fig1: Western blot analysis of ATL3 on different lysates with Rabbit anti-ATL3 antibody (HA721824) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: A549 cell lysate
Lane 4: HepG2 cell lysate
Lane 5: COS-1 cell lysate
Lane 6: NIH/3T3 cell lysate
Lane 7: RAW264.7 cell lysate
Lane 8: Neuro-2a cell lysate
Lane 9: PC-12 cell lysate
Lane 10: L6 cell lysate
Lane 11: Mouse liver tissue lysate
Lane 12: Rat liver tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 61 kDa
Observed band size: 61 kDa

Exposure time: 30 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721824) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

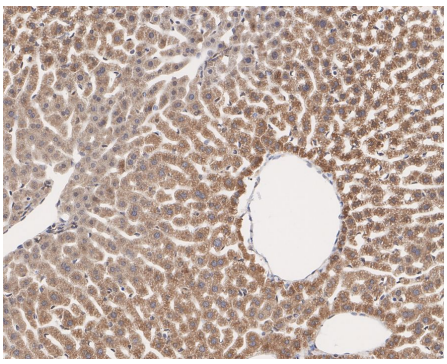

Fig2: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-ATL3 antibody (HA721824) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721824) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

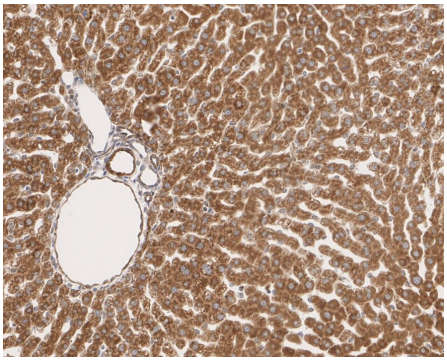

Fig3: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-ATL3 antibody (HA721824) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721824) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

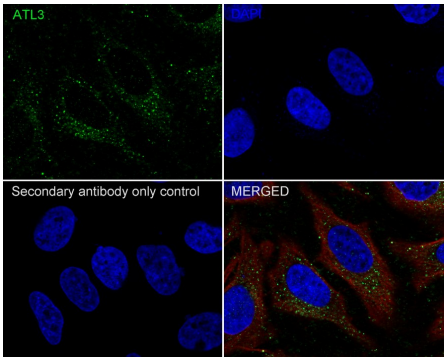

Fig4: Immunocytochemistry analysis of HeLa cells labeling ATL3 with Rabbit anti-ATL3 antibody (HA721824) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-ATL3 antibody (HA721824) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

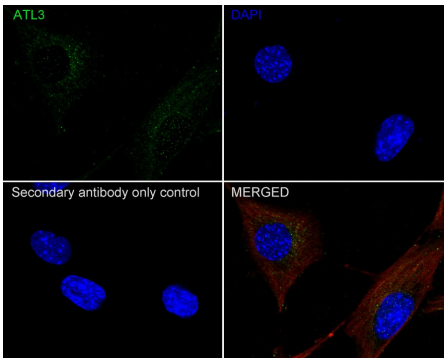

Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling ATL3 with Rabbit anti-ATL3 antibody (HA721824) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-ATL3 antibody (HA721824) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

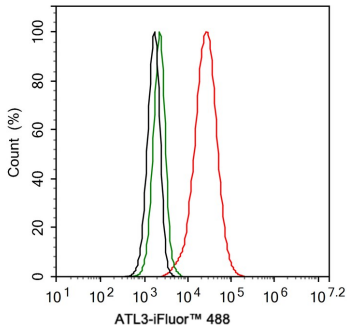

Fig6: Flow cytometric analysis of HeLa cells labeling ATL3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721824, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

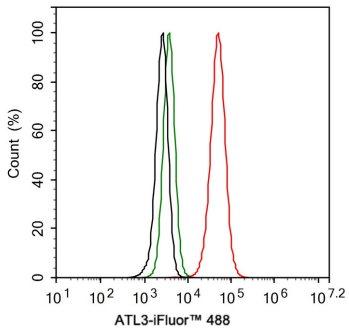

Fig7: Flow cytometric analysis of NIH/3T3 cells labeling ATL3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721824, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

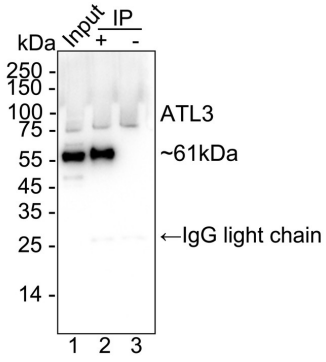

Fig8: ATL3 was immunoprecipitated from 0.2 mg Jurkat cell lysate with HA721824 at 2 μg/10 μl beads. Western blot was performed from the immunoprecipitate using HA721824 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Jurkat cell lysate (input)
Lane 2: HA721824 IP in Jurkat cell lysate
Lane 3: Rabbit IgG instead of HA721824 in Jurkat cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 7 seconds; ECL: K1801

Background References

1. Chen Q et al. ATL3, a cargo receptor for reticulophagy. Autophagy. 2019 Aug
2. Tan X et al. Coronavirus subverts ER-phagy by hijacking FAM134B and ATL3 into p62 condensates to facilitate viral replication. Cell Rep. 2023 Mar